The Role of the Host in the Development of *in vivo* Models for Carcinogenesis Studies*

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ABSTRACT

The development of cancer depends upon the integrated response of the host to the carcinogen and to the initial transformation event. Genetic factors determine the host's potential to respond to chemical and viral carcinogens, while endogenous and exogenous environmental factors influence the realization of the genetic potential. In chemical carcinogenesis, inducibility (the capacity to metabolize polycyclic aromatic hydrocarbons (PAH) to the ultimate carcinogen) has been demonstrated to be under genetic control; however, tumors will occur to a lesser degree and after a longer delay when large doses of PAH are administered to noninducible mice. Aryl hydrocarbon hydroxylase induction by non-carcinogenic materials may also influence the effects of PAH carcinogens.

In viral carcinogenesis, evidence points to genetic transmission of the RNA oncogenic viruses with expression of the viral genome under host control. Host control over RNA tumor viruses may be demonstrated by the existence of epigenetic, xenotropic, and pantropic viruses; permissive, restrictive, producer, and nonproducer cell lines; and the presence of viral group specific antigen, infectious virus, and/or neoplasia in the host.

The development of cancer from the initial transformed cells (chemical and/or viral induced) is dependent on the host response which is influenced by numerous factors. Immunosurveillance is probably the first line of defense against these transformed cells. Genetic control of immunocompetence is evidenced by the variety of responses to various antigenetic stimuli in genotypically different strains of mice. Immunosuppressive effects of carcinogens, drugs, infections, etc., appear to make possible the initial act of establishing clones of transformed cells by overriding the immunocompetence of the host. Other factors related to diet, aging, stress, etc., effect the host control over the carcinogenic event and may be related to the increased susceptibility to carcinogens and/or the increase in the incidence of "spontaneous" tumors.

In our laboratory, we have undertaken studies to provide the best possible mouse model system for studying respiratory carcinogenesis. These studies with inbred mice have included the determination of relative susceptibility to various carcinogens, AHH inducibility, type C

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RNA viral expression, immunocompetence when challenged by various antigens, and the immunosuppressive effects of various chemical carcinogens. We plan to further evaluate our model systems with regard to environmental stress factors, cocarcinogenesis, and viral infections. In this way, we hope to establish the integrated host response to the events occurring in respiratory carcinogenesis.

Introduction

The ultimate solution of the cancer problem is dependent not only on the determination of the causes of cancer but also on the mechanisms whereby the host regulates the prevention and surveillance of the carcinogenic event(s) and the ultimate growth of the cancer. We must now ask what are the differences between susceptible and resistant hosts to the causes of cancer. Cancer, as a disease entity, differs in many respects from infectious diseases, in that there is a greater host-parasite involvement. Evidence points to cancer being an epigenetic disease (i.e., its development is dependent on an innate genetic resistance predisposed to gradual failure of control surveillance mechanisms). However, this is not the entire picture, since the response of the host to carcinogenic and subsequent events is an integrated response dependent on the interactions of both genetically controlled mechanisms and internal as well as external environmental factors.

A prerequisite to the etiological studies of any disease is the development of an appropriate animal model system. With the resolution of this problem, rapid progress in treatment and prevention can generally be made. Perhaps the greatest problem in carcinogenesis research has been the lack of a classical experimental animal model system. MEKLER (1973) has suggested that certain fundamental laws of biology pertinent to carcinogenesis have either escaped the attention of researchers or have not yet been discovered. The great strides which have been made in the study of host factors believed relevant to cancer have led us to the conclusion that we must better understand the role of the host in carcinogenesis before we can judiciously select the best animal model.

What species of laboratory animals provides us with the most information regarding those host surveillance mechanisms that may play a role in carcinogenesis? At this time, one would have to agree that there is more available information for various inbred strains of mice. For this reason, we suggest the mouse is still our best hope for selecting one or more model systems where inbreeding can provide the characterization of genetic factors related to carcinogenic susceptibility. There is probably no greater justification for using rats, rabbits, or for that matter, primates to demonstrate the carcinogenic potential of most chemicals. There are other justifications for the use of mice. Much of our knowledge regarding oncogenic viruses comes from mouse studies and has only recently been expanded to other species. In many ways, because of the broad knowledge of mouse genetics, we can come closer in mice to mimicking the situation that exists in man than in any other animal. Mice are easy to handle and require less space than most other animals; therefore, statistically significant results can be obtained by the use of large populations. Their relatively short life span of 2 to 3 years also makes them ideal subjects for lifetime studies. Their extraneous virus flora have been relatively well characterized and can be controlled by quarantine procedure. Their "spontaneous" tumor incidences have also been characterized.

There are of course various disadvantages, as is true with any model system. For respiratory carcinogenesis studies, mice have the disadvantage of being obligate nose breathers and this may present a different picture from that seen in man. Rats and mice have 4 nasal glands (vs 1 in man) which discharge fluids upon breathing noxious chemical or physical irritants. Mice also have more goblet cells per surface area of the respiratory epithelium (WYNDER and HOFFMANN, 1969). The organs and blood supply in mice are also small, thus presenting the disadvantage that only a limited number of studies can be undertaken with any one animal.

For the past 10 years, our laboratory has been actively defining certain parameters of viral-chemical carcinogenesis. Fortunately, other laboratories have pursued other aspects of genetically controlled surveillance mechanisms, which play important roles in cancer. It is our purpose in this paper to look at the integrated host (i.e., the inbred mouse) in an attempt to pull together many of the factors we feel play a role in the selection of the best possible animal model system for respiratory carcinogenesis. Our discussion of the mouse model system presents several aspects of host control over susceptibility:

- Susceptibility to chemical carcinogens
- Chemical carcinogen metabolism
- Viral etiology of cancer
- Tumor immunology
- Other genetic controls of neoplastic development
- Cells at risk to carcinogens and DNA repair

Chemical Carcinogenesis Susceptibility

During the past 7 years we have concentrated primarily on the characterization of various genotypically different mouse strains as to their susceptibility to 3-methylcholanthrene (MCA), 7,12-dimethylbenz(a)anthracene (DMBA), and benzo(a)pyrene (BaP) when given subcutaneously to female mice 4 weeks of age (WHITMIRE et al., 1971; WHITMIRE and SALERNO, 1972; KOURI et al., 1973b). These studies were based on an 8 month observation period, since most tumors occur during the first 5 to 6 months. The doses selected were relatively small, since our primary aim was to determine relative susceptibility in a reasonable period of time with as few adverse effects on the host as possible. The basis for comparison has been the cumulative tumor incidence, latency period (SHIMKIN and ANDERVONT, 1940), carcinogenic index (CI), (% cumulative tumors divided by the average days latency X 100) (IBALL, 1939), and tumor inducing dose in 50% of the animals in the defined observation period (TuD_{50}), (REED and MUENCH, 1938). We studied those mouse strains (inbred and random bred) most frequently used in cancer research. We have found correlation of the extensive literature difficult due to the numerous variables that inevitably exist between laboratories. Our results from studies carried out over an extended period using the same techniques, carcinogen dose, vehicle, and mice of comparable age and sex are presented in Table 1. Table 2 gives some of the host genetic factors for these inbred strains as a ready reference for later discussions.

These earlier studies with MCA have demonstrated a wide variation in the tumor incidence as well as average latency period among the genotypically different strains. Although the most susceptible strains generally develop tumors in the least amount of time, this is not

necessarily the case. For this reason the carcinogenic index (CI) has been utilized as a valuable index in rating the relative susceptibility of the various mouse strains (IBALL, 1939; WHITMIRE and SALERNO, 1972a). Probably the most outstanding examples of variation between tumor incidence and latency are the C3H/fMai and the C3H/HeJ, whose average latency is 14 weeks in each incidence but which produce 93% and 62% tumors respectively. Another example is that of the C3H/HeJ mice (14 weeks latency), which produce only 62% tumors, and the C57BR/cdJ strain, which produce 67% tumors with a relatively long average latency (22 weeks). These studies demonstrate that, although NIH Swiss, Ha/ICR, CFW, and CF-1 random bred strains have been used extensively in carcinogenesis studies, they are not as susceptible as a number of inbred strains. To define the relative susceptibility to MCA carcinogenesis still further, several dose levels were used and we found the C3H/fMai mouse strain to be consistently more susceptible than the other strains tested (Table 3). These studies also demonstrate a difference in mice of the same strain obtained from different sources. This is one variable in carcinogenesis studies of which most investigators are not aware and undoubtedly accounts for some of the variations in reported findings from one laboratory to another. The C57BL/6 mice from 3 sources act as entirely different strains (WHITMIRE and SALERNO, 1972a).

Table 1. Subcutaneous chemical carcinogenous characterization of various mouse strains with 150µg MCA

MOUSE STRAIN	TUMOR INCIDENCE Tu/T	%	AV. TU. LATENCY (WKS)	C.I.[a]	AHH[b] INDUCIBILITY	gs-1 ANTIGEN INCIDENCE P/T[c]	%	INFECTIOUS TYPE-C VIRUS
INBRED								
C3H/f Mai	51/55	93	13	102	9	16/23	70	±
C57BL/6 Cum	18/20	90	17	76	10	3/26	12	–
C58/J	20/23	87	16	76	10	10/10	100	+++
C57BL/10ScSnJ	22/27	81	18	64	12	0/14	0	–
BALB/cCr (Mai)	35/49	71	19	53	8	5/23	22	±
C57BR/cdJ	18/27	67	22	44	7	2/18	11	–
C3H/HeN	33/53	62	14	63	10	13/24	50	+
SWR/J	11/22	50	23	33	1	6/10	60	–
129/J	14/30	47	27	25	1	0/15	0	–
C57L/J	13/28	46	20	33	6	0/13	0	–
AKR/J	20/51	39	26	21	1	9/9	100	+++
SJL/J	13/35	37	22	24	1	11/15	73	++
DBA/2J	5/35	14	28	7	1	12/12	100	+
DBA/1J	6/53	11	27	6	1	4/8	50	+
RANDOM BRED								
SWISS-WEBSTER (N)	26/35	74	17	62	1	13/25	52	–
Ha/ICR (RPMI)	46/63	73	19	55	1	14/23	61	++
CFW	21/29	72	17	61	1	9/13	69	+
CF-1	37/66	56	23	33	1	15/15	100	++
SNELL/Mai	9/25	36	24	14	1	11/15	73	+

[a] CARCINOGENIC INDEX = $\frac{\%\ \text{TUMORS}}{\text{AV. DAYS LATENCY}} \times 100$

[b] AHH INDUCIBILITY = $\frac{\text{AHH LEVELS FROM MCA-TREATED MICE}}{\text{AHH LEVELS FROM TRIOCTANOIN TREATED MICE}}$

HEPATIC AHH LEVELS WERE DETERMINED 24 HOURS AFTER IP ADMINISTRATION OF 80 µg MCA/g BODY WEIGHT OR 0.05 ml TRIOCTANOIN. CONSTITUTIVE AHH LEVELS WERE SIMILAR FOR EVERY STRAIN TESTED

[c] $\frac{P}{T} = \frac{\text{NUMBER OF POSITIVE SAMPLES BY CF TEST}}{\text{TOTAL SAMPLES TESTED}}$

Table 2. Genes influencing AHH inducibility, tumor virus histocompatibility, and immunological responses

MOUSE STRAIN	GENE DESIGNATION[a]						IMMUNOLOGICAL RESPONSES[b] H-2 ASSOCIATED						NON H-2 ASSOCIATED			
							Ir-1 GENES SYNTHETIC POLYPEPTIDE COPOLYMERS (,)-A--L			GAT$_{10}$	Ir-Ig GENES BALB/c MYELOMA PROTEIN		Ir-3 GENES POLYPEPTIDES (,)-Pro-L		SC[c]	
	Ah	Fv-1	Fv-2	H-2	Ir-1	Ir-3	(T,G)	(H,G)	(Phe,G)		IgG	IgA	(H,G)	(T,G)	A	C
C3H/f	b	n	s	k	a	H9	L	H	H	H	L	H	H	H		
C57BL/6	b	b	r	b	b	H9	H	L	H	H	H	L	H	M		
C58	b	n	r	k	a	H9	L	H	H	H	L	H				
C57BL/10ScSn	b	b	r	b	b	H9	H	L	H	H	H	L				
BALB/c	b	b	s	d	a	H9	Mv	Mv	H	H	L	L				
C57BR/cd	b	n	r	k	a	H9	L	H	H	H	L	H				
C3H/He	b	n	s	k	a	H9	L	H	H	H	L	H	H	L		
SWR	d	n	s	q	c	H9	L	L	H		L	L	H	H	H	H
129	d	n	s	b	a	H9	H	L	H	H	H	L				
C57L	b	n	r	b	a	H9	H	L	H	H	H	L				
AKR	d	n	s	k	d	H-	L	H	H	H	L	H	H	L		
SJL	d	n	s	s	b	H9	L	L	L	neg	H	H	H	H	H	M
DBA/2	d	n	s	d	c	H9	Mv	Mv	H	H	L	L	H	L	H	M
DBA/1	d	n	s	q	c	H9	L	L	H		L	L	L	L	H	L

a STATTS, 1972

b McDEVITT AND LANDY, 1972

L = LOW
M = MEDIUM
Mv = MEDIUM VARIABLE
H = HIGH

[c]STREPTOCOCCAL CARBOHYDRATE ANTIGENS

Table 3. Comparison of tumor incidence, mean latency, CI and TuD_{50} in various strains of mice treated subcutaneously with various doses of MCA at 4 weeks of age

MOUSE STRAIN	9.38 µg TUMOR INCIDENCE Tu/T	%	MEAN CI LAT (WKS)		37.5 µg TUMOR INCIDENCE Tu/T	%	MEAN CI LAT (WKS)		150.0 µg TUMOR INCIDENCE Tu/T	%	MEAN CI LAT (WKS)		TuD_{50} µg MCA
AHH INDUCIBLE													
C3H/fMai	9/28	32	23	20	24/29	83	18	68	27/30	90	13	96	21
C3H/AnfCum	9/29	31	21	21	16/29	55	17	46	25/29	86	14	89	57
C57BL/6Cum	10/28	36	21	21	14/27	52	23	32	26/27	96	17	83	26
C57BL/6Mai	–	–	–	–	12/30	40	21	28	20/27	74	19	50	61
C57BL/6J	–	–	–	–	14/29	48	20	34	16/27	59	20	44	64
BALB/cCR (Mai)	3/30	10	27	7	17/28	61	20	45	23/28	82	16	71	34
BALB/cSPF (Mai)	–	–	–	–	20/30	67	17	57	26/31	84	12	98	≥38
C57BL/10ScSn	–	–	–	–	12/30	40	23	25	22/27	81	18	64	41
AHH NON-INDUCIBLE													
	150.0 µg				300.0 µg				500.0 µg				
129/J	14/30	47	27	25	16/28	57	21	39	20/28	71	22	46	203
DBA/2J	10/24	42	25	24	14/24	58	22	38	21/24	88	21	59	212
DBA/1J	4/27	15	25	9	13/24	62	25	35	10/19	53	23	33	238

The susceptibility of an animal to one carcinogen does not insure its responsiveness to another carcinogen. Therefore, studies were undertaken with DMBA and BaP in selected strains. As seen in Table 4, the C3H/fMai strain is highly susceptible to all 3 carcinogens, while the other strains are relatively insensitive to tumor induction with BaP and show varying sensitivity to DMBA. In all instances, the latency periods were longer with DMBA and BaP than with MCA. Although all polycyclic aromatic hydrocarbons are believed to be metabolized by the AHH enzyme system, there is evidence that their metabolism pathways are slightly different (NEBERT et al., 1973), which may account for genetic differences in the strains tested.

Chemical Carcinogen Metabolism

In order for polycyclic aromatic hydrocarbons (PAH) to exert cell transformation, mutagenicity, and tumor induction, they must be metabolized to water soluble active forms (e.g., the epoxide), (MARQUARDT and HEIDELBERGER, 1972; HUBERMAN et al., 1972) by the microsomal bound, mixed function oxidases of which aryl hydrocarbon hydroxylase (AHH) enzymes are a major system found in the tissues of man (KELLERMANN et al., 1973) and animals (GELBOIN, 1967). Constitutive enzyme levels are normally detectable without induction; however, the inducibility of AHH is associated with the carcinogenic effects of PAH (SELKIRK et al., 1971). The AHH system is inducible by a variety of endogenous chemicals (corticorsteroid hormones and bilirubin), as well as exogenous chemicals (barbiturates, insecticides, and PAH); therefore, these enzymes obviously may function as a two-edged sword. Our studies with various strains of mice have demonstrated a direct correlation between inducibility of AHH activity and 150µg MCA subcutaneous carcinogenesis (KOURI et al., 1973b). The results are summarized in Table 1. If, however, one gives higher doses of MCA (Table 3) to noninducible mice, the incidence of tumors can be increased, while the latency period remains at 5 to 6 months and the CI index below 60. The TuD_{50} dose is 3 to 11 times that of the inducible mice.

The role of AHH appears highly specific for each PAH. Thus, the metabolism of MCA, DMBA, and BaP does not necessarily follow the same pathways indicated by subcutaneous and skin carcinogenesis studies (KINOSHITA and GELBOIN, 1972; KOURI et al., 1973b; NEBERT et al., 1973). These differences in the carcinogenic effects (Table 4) indicate that the C3H/f mouse was the only strain capable of handling all 3 carcinogens equally well. It would appear that these variations in the AHH system could be clarified by studying congenetic crosses between the C3H/f strain and another strain giving low levels of tumor induction with BaP and DMBA and might help explain important etiological differences in chemical carcinogenesis.

The inducibility of the AHH system by various chemicals has been shown to be under host regulation. Susceptibility to MCA induction segregates as a single autosomal dominant gene in crosses involving the C57BL/6 (B6) and DBA/2 (D2) strains of mice (THOMAS et al., 1972; NEBERT et al., 1972b; GIELEN et al., 1972b). The B6 is the prototype inducible strain and its allele Ah^b designates the dominant gene. The D2 strain is the prototype strain for the recessive Ah^d allele. We utilized this mouse genetic system to extend our observations on the relationship between AHH inducible and sensitivity to MCA tumorigenesis (KOURI et al., 1973a, 1974c). The results in Table 5 demonstrate that inducible animals were approximately 10 times more sensitive to MCA carcino-

Table 4. Tumor incidence, mean latency, and CI in various strains of mice (females) inoculated subcutaneously with 150µg MCA, DMBA and BaP

	150 µg MCA				150 µg DMBA				150 µg BaP			
MOUSE STRAIN	Tu/T	%	MEAN LAT (WK)	CI	Tu/T	%	MEAN LAT (WK)	CI	Tu/T	%	MEAN LAT (WK)	CI
C3H/fMai	51/55	93	13.2	100	24/25*	96	17.5	78	20/24*	83	18.3	65
B10.BR/J	26/28	93	16.3	83	16/29	55	21.0	37	3/25	12	21.7	8
C57BL/6Cum	18/20	90	16.5	78	5/17	29	22.4	19	2/24	8	19.0	7
C57BL/10ScSn	22/27	81	18.1	64	10/24	42	22.8	26	4/29	14	24.0	8
129/J	14/30	47	27.0	25	11/33	33	26.5	18				
Snell/Mai	9/25	36	23.6	24	8/23	35	24.0	29	3/29	10	22.0	6

*THESE ANIMALS WERE MALES

genesis than noninducible animals when comparing the CI values. In every case where a tumor was observed on a noninducible animal, it occurred late in the observation period after tumor development had ceased in the inducible animals. It is assumed that some metabolism of this carcinogen took place at a much slower rate (possibly by the constitutive levels of AHH enzymes), leading to cell transformation and tumor induction.

The significance of AHH induction in the transformation of tissue culture cell lines by PAH carcinogens has been demonstrated (KOURI et al., 1974b). Only those cell lines potentially sensitive to chemically induced transformation possessed the particular type of metabolism involving the AHH inducible enzymes. The carcinogenic effect of a hydrocarbon is probably determined by the amounts activated to the carcinogenic form. The low levels of these enzymes in many tissue culture cell lines are probably a factor in the inability to obtain chemically induced transformation.

Enhancement and interference in the metabolism of chemicals by the AHH system can occur. CONNEY (1974) has demonstrated that oral treatment with MCA, DMBA, and BaP enhanced the metabolism of intravenously (IV) administered radioactive BaP in rats, while phenobarbital (which stimulates AHH induction) did not enhance MCA, BaP, or DMBA metabolism. Chronic administration of BaP stimulates the metabolism of radioaction BaP. These observations are of interest since treatment of rodents with AHH inducers provides protection for the carcinogenic effects of BaP, DMBA, N-2-fluorenylacetamide, 4-dimethylaminostibene, urethane, aflatoxin, diethylnitrosamine, and aminoazo dyes (CONNEY, 1974). THAMAVIT et al. (1974) have also reported that treatment with 3 carcinogens at one time decreases their carcinogenesis in rats and was believed due to an interference phenomena. WEBER et

Table 5. Relationship of hepatic inducibility and subcutaneous tumor induction with 150µg MCA in C57BL/6 x DBA/1, F1, and F2 mice

	AHH INDUCIBLE		AHH NON-INDUCIBLE	
	TUMOR INCIDENCE		TUMOR INCIDENCE	
MOUSE STRAINS	Tu/T	%	Tu/T	%
AHH NONSEGREGATING:				
B6 (Ah^b/Ah^b)	23/29	79		
D2 (Ah^d/Ah^d)			2/30	7
F1 (Ah^b/Ah^d)	54/90	60		
F1 x B6 (Ah^b/Ah^d and Ah^b/Ah^b)	81/94	86		
TOTALS:	158/213	74	2/30	7
AHH SEGREGATING:				
F1 x D2 (Ah^b/Ah^d and Ah^d/Ah^d)	15/24	75	5/34	15
F2 (Ah^b/Ah^b Ah^b/Ah^d Ah^d/Ah^d)	23/25	92	2/21	10
TOTALS:	38/45	84	7/55	13
TUMOR TOTALS:	196/258	77	8/85	11
AV. DAYS TUMOR LATENCY:	131		195	
CARCINOGENIC INDEX:-	59		6	

al. (1974) recently reported that nicotine reduced the metabolism of benzopyrene in tobacco smoke thus demonstrating interference between chemicals metabolized by the same enzyme system.

Not all known carcinogens belong to the group of chemicals known as PAH and their metabolism proceeds along different pathways. They have not been studied as extensively as the PAH group and the relationship to genetic patterns of susceptibility to carcinogenesis has not been determined. The dimethylase associated with the metabolism of the dimethylnitrosamines (DMN) may bear a mirror-image relationship to the AHH system, since high levels of induced AHH may act as potent repressors of DMN-dimethylase activity (VENKATESAN et al., 1971). This suggests that DMN may be a more potent carcinogen in AHH non-inducible animals. We are undertaking studies in AHH inducible and noninducible animals to test this hypothesis.

Virus Etiology of Cancer

Although a number of tumor viruses have been isolated from a variety of animal species since ROUS (1911) first discovered the avian sarcoma virus, the concept of viral etiology of cancer has been untenable for many scientists. The development of inbred mouse strains produced high and low incidence leukemia strains and ultimately led to the demonstration that RNA tumor viruses could be transmitted both horizontally and vertically (GROSS, 1944, 1950, 1970). Transmission can take place by congenital infection of the germ cells or via the placenta or milk; however, the usual mode of spread appears to be by genetic inheritance from one generation of animals or cells to the next as DNA copies of viral RNA integrated into the genetic material of the cells (WEISS, 1973; BUFFET et al., 1969; HILGERS et al., 1972).

Two concepts have been proposed for the origin of RNA tumor viruses: the oncogene hypothesis of HUEBNER and TODARO (1969) (TODARO and HUEBNER, 1972) and the protovirus hypothesis of TEMIN (1971, 1972). The oncogene theory suggests that viral genetic material is present in normal cells expressed as infectious virus or as noninfectious viral subunits (as viral group specific (gs) antigen) and that a noninfectious portion of the viral genome (the oncogene) is responsible for cellular transformation and cancer. The protovirus hypothesis differs from the oncogene hypothesis by suggesting that infectious viral genetic information is transferred by transcription and reverse transcription and, in combination with mutation or recombination events, produces neoplastic transformation.

Regardless of the hypothesis for the origin of the RNA tumor viruses, there are certain cellular controls which govern their expression. These controls will vary somewhat between endogenous and exogenous viruses; therefore, it is pertinent that some of the properties of each be considered. Endogenous viruses are transmitted vertically, either as viral genome or as infectious virus by congenital means. Multiple copies of the virogene are present in the DNA of all somatic and germ cells of all animals in a species. The type of viral expression is under cellular control and may be present as gs antigen, defective virus, or complete virus capable of growth under proper conditions with the production of reverse transcriptase (RT). Clonal lines established from these tissues will either spontaneously release virus or induce virus release after varying intervals of cultivation, depending on the original viral expression and the strength

or degree of cellular control. Induction can also be accomplished with 5'-iododeoxyuridine (IudR) or 5-bromodeoxyuridine (BudR). Complete virogene is known to be present in chickens, Chinese and Syrian hamsters, mice, rats, cats, pigs, and baboons and has been demonstrated by single cell clones with release of infectious virus. These cells with endogenous virogene are generally resistant to exogenous infection by the homologous endogenous virus. The expression of endogenous virus is influenced by the genetic properties of the virus and the cell as well as exogenous factors, such as radiation, chemical carcinogens, etc. Exogenous viruses differ from endogenous viruses in that they are spread horizontally as infectious virus (with evidence of RT) from animal to animal or cell to cell.

The characterization of the mouse type C viruses has recently been reviewed by SARMA and GAZDAR (1974). The type C RNA viruses from mice can be divided into 2 groups. The sarcoma viruses (MSV) produce solid tumors *in vivo* and transformed or cytopathogenic foci *in vitro*. These have been isolated rarely from laboratory adopted stocks of mouse leukemia viruses (MuLV), (HARVEY, 1964; MOLONEY, 1966; KIRSTEN and MAYER, 1971) or from several spontaneously occurring mouse sarcoma (FINKEL et al., 1966; GAZDAR et al., 1972). MSV can transform cells and release virus or they can fail to release virus, as seen in the nonproducer cell lines where the viral genome is integrated into the genetic material of the host cell. In such cases the viral genome can be rescued by superinfection with MuLV, which provides the envelope for the defective MSV. The mouse leukemia viruses (MuLV) are noncytopathogenic *in vitro* when propagated in permissive cells. *In vivo*, some produce leukemia while others fail to produce evidence of any neoplastic potential under the test conditions. They have been isolated from spontaneous and chemically induced solid tumors.

Recently, it has been shown that 2 classes of murine, RNA, type C viruses exist, based on their ability to replicate in mouse tissue. Those which will not replicate in mouse tissue but require rabbit, human, cat, etc., tissue are called xenotropic viruses (X-tropic), (LEVY, 1973) or S-tropic (SHERR et al., 1974). These viruses have typical murine type C antigenic markers but differ distinctly by nucleic acid hydridization from the N-tropic MuLV (BENVENISTE et al., 1974). The significance of the X-tropic viruses has not been determined at this time although they are apparently widespread. Their presence in the mouse with the apparent inability to propagate at least *in vitro* in mouse tissues presents an interesting type of genetic control that requires additional study. The implications of such viruses in humans provide a possible explanation to our inability to propagate a human cancer virus. MuLV, which replicate preferentially in mouse tissues, have been classified as ecotropic viruses (LEVY, 1974), and make up the group of murine viruses for which considerably more information is available. It is these viruses that will be discussed in this paper.

Vertical transmission of virogene information bypasses the host controls associated with infectious viral (but not necessarily oncogene) expression. Such host controls over infectious virus are common to vertically and horizontally transmitted infectious virus expression. Evidence of viral genetic information in the absence of infectious virus has been demonstrated by several systems. Virus-like molecule sequences were reported by HAREL et al. (1967) in the DNA of uninfected murine cells. CHASE and PIKO (1973) and VERNON et al. (1973) observed C-type particles, and gs antigen (HUEBNER et al., 1970a) has been demonstrated in embryonic tissues of mice. The $Gross_{IX}$ MuLV associated antigen was demonstrated by STOCKERT et al. (1971). Spon-

taneous and induced appearance of MuLV from clones of nonproducer cell lines has been demonstrated by TODARO (1972), AARONSON et al. (1969, 1971).

Based on our present technology for detecting type C RNA viral expression there appear to be 4 categories of mouse strains: 1. the C57L mouse expresses no viral antigen or infectious virus; 2. the NIH Swiss mouse has only the gs-1 antigen and no G_{IX} antigen, and the 129 strain expresses some gs-1 and G_{IX} antigen (neither of these 2 mice has infectious ecotropic MuLV, although type C particles have been observed in NIH Swiss mice); 3. certain mice have a low incidence of early ecotropic MuLV expression as gs-1 antigen that gradually increases and is accompanied by low levels of infectious MuLV expression as seen in the BALB/c mouse (PETERS et al., 1972a); and 4. a high level of infectious virus is detected early in life, accompanied by a high incidence of leukemia as demonstrated in AKR, C58, and C3H/Fi.

Several genes have been associated with endogenous virus expression; however, their roles have not been fully evaluated and may be related more to the expression of neoplasia than the virus per se. The TL antigen, determined by the Tla locus, may represent a viral genome since it appears only on leukemic cells and thymocytes of certain strains (BOYSE and OLD, 1969). The G_{IX} antigen is found on thymocytes and lymphocytes, but all murine leukemic cells are not G_{IX}^{+} (STOCKERT et al., 1971). TAYLOR et al. (1971) described 2 independent genes (no designation made) for gs antigen expression in the AKR mouse and postulated one locus for gs antigen expression and another for infectious virus in the AKR X C57BL/6 F2 cross. It is possible these 2 genes may be the same as the V_1 and V_2 loci for complete virus production in the AKR mouse described by ROWE (1972). These V-loci and the Ind locus (STEPHENSON and AARONSON, 1972a, 1972b) predispose cells to virus induction by IudR and BudR (ROWE et al., 1971). TAYLOR et al. (1973) also described the Mlv-1 allele in the C57BL/10 and DBA/2 strains as a determinate of gs antigen expression. Another gene (Fv-2) has been characterized for host control over propagation of the spleen focus-forming virus (SFFV) component of Friend virus complex. The susceptibility phenotype ($Fv\text{-}2^{s}$) is dominate and the $Fv\text{-}2^{r}$ denotes absolute resistance. This linkage is unrelated to the H-2 linkage and has no direct influence on the lymphatic leukemia virus of Friend (MCDEVITT and LANDY, 1972).

Interferon has been shown to be a potent antiviral agent and its production has been shown to be under host control (deMAEYER and deMAEYER-GINGNARD, 1969). This control is apparently related to the host response to various interferon inducers (BARON, personal communication, 1974) therefore demonstrating another variation in host control of infectious virus expression.

The best defined of the host cellular control genes for MuLV is that governing the replication of infectious virus. This spreading factor influences the ability of endogenous as well as exogenous ecotropic viruses to express themselves as infectious virus. The Fv-1 locus controls the host range permissiveness of mouse cells for the replication of MuLV (PINCUS et al., 1971a, 1971b). HARTLEY et al. (1970) demonstrated 3 groups of MuLV based on their ability to grow more efficiently in NIH Swiss (N) cells ($Fv\text{-}1^{n}$) or BALB/c (B) cells ($Fv\text{-}1^{b}$) and designated this predilection of the viruses as "N- and B-tropic" and NB-tropic" for those viruses that grow equally well in both types of cells. The tropism of the various mouse strains and their embryonic tissue culture cells have been classified as N-type or B-type. In the case of the MSV, the tropism is dependent on the tropism of the helper

virus. Mice strains that develop early leukemia belong to the Fv-1^n group (AKR, C58, C3H/Fi). These mice also carry the V_1 and V_2 alleles for high incidence activation of the endogenous MuLV. See Table 6 for summary of host genes associated with RNA type C viral expression control.

These type C viruses and the various host control mechanisms have been shown to play a role in the incidence of "spontaneous" (PETERS et al., 1972b) and virally induced neoplasia (LILLY and PINCUS, 1973; ROWE, 1972). They have been postulated to be switched on by chemical carcinogens (HUEBNER et al., 1970b, 1972; MEIER and MYERS, 1973); however, their significance in chemical carcinogenesis is still open to speculation. If one does undertake induced viral-chemical carcinogenesis studies, one must be cognizant of the susceptibility of the mouse strain not only to the chemical carcinogen used but also to the virus selected for these studies. It is also necessary to recognize the importance of endogenous viruses and the ensuing natural incidence of early or late development of leukemia when selecting inbred strains and congeneic strains for carcinogenesis studies related to the interaction of spontaneous and induced neoplasia (WHITMIRE et al., 1972b, 1972c, 1973a; SALERNO et al., 1973).

To determine the occurrence and concomitants of viral expression during MCA carcinogenesis in the various mouse strains, we have followed the incidence of gs antigen and infectious virus in the induced tumors (WHITMIRE et al., 1971, 1973b; WHITMIRE and SALERNO, 1972a). These results are summarized in Table 1. There is no significant correlation between susceptibility to MCA and the presence of viral expression as gs antigen or infectious virus. Other studies have confirmed this finding with DMBA and BaP (KOURI et al., 1973b). The gs antigen expression in chemically induced tumors follows the same pattern as that of the spleens of normal animals (MYERS et al., 1970) and could be related to the degree of expression that increases with age, as observed in the BALB/c mouse (WHITMIRE et al., 1973b).

Tumor Immunology

The significance of the host's ability to respond immunologically to the events of carcinogenesis was recognized early, yet today not all the ramifications of tumor immunology are understood. Further advances in technology and more knowledge regarding the integrated nature of host defense mechanisms might shed some light on this complex area. There exists the dichotomy between the healthy immune stimulus that provides for elimination of the initial transformed cells or the holding action in the host-parasite relationship, and the unhealthy condition where the immunological response enhances tumor growth. A point in cancer research has been reached when we must use the available knowledge regarding immunological competence for the selection of our animal models. It is my purpose to review some of these factors to be defined or at least recognized as existing in the animal models selected for the study of viral, chemical, and viral-chemical carcinogenesis. Sweeping conclusions regarding carcinogenic events can no longer be made in model systems without considering the integrated host reactions.

The metabolism of chemical carcinogens and factors related to viral etiology have already been considered; however, it will be necessary to consider the immunological response involved in those 2 facets that

Table 6. Genes influencing ecotropic mouse tumor virus expression (TOOZE, 1973)

ALLELE	PHENOTYPE	EXPRESSION	EXAMPLE STRAIN	REFERENCE
Tla	TL ANTIGEN	+	A, C58	BOYSE & OLD (1969)
		−	AKR, C57BL/6	
NOT DESIGNATED	gs ANTIGEN	+	AKR	TAYLOR, MEIER, MYERS (1971)
		−	C57L	
NOT DESIGNATED	INFECTIOUS MuLV	+	AKR	TAYLOR, MEIER, MYERS (1971)
		−	C57L	
V_1	N-TROPIC MuLV INDUCTION	V_1	AKR, C58, C3H/Fi	ROWE (1972)
		v_1		
V_2	N-TROPIC MuLV INDUCTION	V_2	AKR, C58, C3H/Fi	ROWE & HARTLEY (1972)
		v_2		
Ind	N-TROPIC MuLV INDUCTION	+	BALB/c	STEPHENSON & AARONSON (1972b)
		−	NIH-SWISS	
Fv-1	TISSUE TROPISM FOR VIRAL REPLICATION	n	AKR, C58	ROWE & HARTLEY (1972)
		b	C57BL/6, BALB/c	STEPHENSON & AARONSON (1972a)
		nb	NZB	
H-2	EARLY LEUKEMIA	k	AKR	BOYSE, OLD, STOCKERT (1972)
	LATE LEUKEMIA	b	AKR/H-2^b	
G_{1X}	G_{1X} ANTIGEN	+	129	BOYSE, OLD, STOCKERT (1972)
		−	C57L	
Mlv-1	gs ANTIGEN	a	C57BL/10, DBA/2	TAYLOR, MEIER, HUEBNER (1973)
		b		

allow the initial carcinogenic event to establish itself. Immunosuppressive effects of virus (SHEARER et al., 1973) and chemical carcinogens (STJERNSWÄRD, 1965; REES and SYMES, 1973; MATSUOKA et al., 1972; PARMIANI et al., 1971; BALL et al., 1966; BALL, 1970) undoubtedly play a significant part in the initial carcinogenic event. We are currently addressing ourselves to defining the immunosuppressive effects of various chemical carcinogens given intratracheally for the induction of lung cancer in several strains of mice (DEMOISE et al., 1974). Just as NETTESHEIM and HAMMONS (1971) have shown differences in susceptibility to lung carcinogenesis by MCA, STUTMAN (1969) indicates differences in the immunosuppressive effects in genotypically different strains. Based on our studies with MCA we would anticipate that both differences in tumor induction and immunosuppression may be correlated with the AHH inducibility of the various mouse strains (KOURI et al., 1973b).

The possible mechanisms whereby chemical carcinogens bring about carcinogenic events may be dependent not only on the transforming events but also on immunodepression, that allows these transformed cells to bypass the immunological defense mechanisms of the host. Immunosuppression by chemical carcinogens may, in fact, not be a single event, but cumulative, recurring events that allow frequent bypassing of host defense mechanisms and consequently slow interrupted but progressive growth of tumor cells.

The term "immunologic surveillance" coined by THOMAS (1959) and further expounded by BURNET (1970a, 1970b) denotes the idea of "seeking out and destroying" transformed cells by immunological means. This idea may not be totally correct, because rather than destruction, a "holding action" (LAPPE, 1971, 1972) may develop until such time as events in the integrated host's defense provide a favorable climate for the growth of the transformed cells (HESTON, 1963). This "sneaking through" event of some transformed cells that ultimately leads to the development of cancer is more of apparent scientific basis than it initially appeared. "Sneak through" has been thought to occur due to the location of the transformed cells in sites not exposed, or exposed less frequently to concomitant immunity. This unequal exposure to immune mechanisms is also believed to play a role in the site of metastites (VAAGE et al., 1971).

Another factor allowing "sneak through" of initial transformed cells is the low antigenic profile of some tumor cells as well as the low antigen load presented by only a few cells. For tumor cells to escape immune surveillance there must first be a barrier to escape. Non-immunogenic or low immunogenic tumors may not be capable of surveillance by immunological means. One must then define what we mean by "nonimmunogenic". Is it that these tumor cells are so like normal cells that they cannot be recognized as foreign by the host? Self-non-self discrimination is not fully understood and is intertwined with immunological tolerance, immunological paralysis, and immunological recognition. Is it that the host is tolerant to these cells since they contain certain embryonic antigens or endogenous viral genome antigens? Or, is it that the host is not capable of recognizing these antigens due to genetic variations within the species? This is where the inbred laboratory animals help us understand the role of genetic determinants in the immune process and, ultimately, to understand the events of neoplastic initiation and developments. Genetic variation in the immunological response can definitely influence neoplasia. Many of the differences in specific immunological responses have been linked to the histocompatibility gene in the mouse, rat, and guinea pig. Viral susceptibility has also been closely associated with the H-2 locus and such variations in susceptibility may be shown to be related, at least

in part, to inability to produce an immunological response in susceptible mice. These immune responses are under the control of individual dominant autosomal genes. Differences in response are not usually all or none but are concerned with quality and/or specificity of the antibody response.

The Ir-1 locus is closely linked to the H-2 gene in mice. Evidence indicates responsiveness to the various peptides (L-tyrosine and L-glutamic acid [T,G], L-phenylalanine and L-glutamic acid [P,G], and L-histidine and L-glutamic acid [H,G] built on multichain poly-DL-alanine) are under the control of different Ir-1 alleles (MCDEVITT, 1968). All of these polypeptides are antigenic in some strain of mice, but not necessarily in any one strain. They will not cross-immunize although antibody will cross react extensively. The Ir-1 gene functions at the T-cell level and controls cellular immune functions in the recognition of antigens, thus low responders are those who have reduced numbers of detectable precursor cells or cells with lower affinity for the specific immunogens. Low responder animals can, however, recognize these polypeptide antigenic determinants and produce large amounts of antibody when these hapten polypeptides are coupled with an immunogenic protein carrier. This shows one means of bypassing genetic defects (MCDEVITT, 1968).

The Ir-1 genes appear to have at least 2 or 3 separate loci. The Ir-IgA gene controls the immune response of mice to allotypic and idiotypic determinants on the IgA myeloma proteins derived from BALB/c mice. The Ir-IgG gene is linked to different H-2 specificities than the Ir-IgA and controls the immune response to γG (γ2a) of the same antigen (LIEBERMAN and HUMPHREY, 1971, 1972).

Various immunological responses have been shown to be non-H-2 linked. In immune responses to the (T,G) or (Phe-G)-Prol--L portion of multichain synthetic polypeptides, the response is controlled by the dominant, autosomal, Ir-3 gene (MOZES et al., 1973). The SJL mouse is the prototype for the high responders and the DBA/1 for the low responders. These responses are expressions of B cell activity and demonstrate antibodies can be made to 2 determinants on the same antigen under the control of 2 genes. Other non-H-2 linked gene controls of immune responses are reviewed by MCDEVITT and LANDY (1972), demonstrating dependence on the recognition of the antigenic determinant.

Antibody response to streptococcal polysaccharides differs dramatically with some strains, producing more to the group A than the group C carbohydrates, whereas in others, the reverse is true. These responses can give rise to a rather homogenous or a wide variation in the heterogeneity of the immune responses. B cell dependent responses to Salmonella lipopolysaccharides have also shown differences in mouse strain response and are dependent on recognition of the antigen (PAULI, 1972).

Having reviewed some of these various aspects of immunogenetic responses, one finds that new scientific discoveries are being made at a rate and volume beyond our ability to assimilate, evaluate, and make use of them as building blocks for rational progress. Unequal progress in the research prevents total integration of this knowledge into the understanding of host-parasite relationships. These studies with natural and synthetic antigens have demonstrated immunogenetic differences in responses which can not at this time be correlated with the viral and tumor specific membrane antigens. Recent advances in characterizing the amino acid sequences in the tumor virus (OROSZLAN et al., 1970; NOWINSKI et al., 1972) and the mouse myeloma protein (FRANCIS et al., 1974), the isolation of the viral envelope glycoproteins (KENNEL et

al., 1973), and studies with carcinoembryonic antigens (TOMITA et al., 1974) will lead to a greater understanding of their immunogenetic potential. Each host responds to some of these antigens but probably not to all of the exposed antigenic configurations making up the tumor cell membranes and soluble antigens. The immunological reactions summarized in Table 2 for the various mouse strains in our carcinogenic studies amplify the subtle difference in their responses to various defined immunogens. Those differences in the capabilities of the host to mount an adequate response to produce a "killer" or "holding" effect influence the capabilities of these genotypic strains to allow "sneaking through" events to occur for the establishment of the initial carcinogenic event as a pathologic entity and also to influence the process of metastasis and the blocking phenomenon (HELLSTRÖM and HELLSTRÖM, 1970) that accompanies rapid growth and terminal events of the neoplastic process. The genetic capabilities of the host to respond may be one of the important factors governing the variation in latency period of tumor development in the animal models as demonstrated in Table 1.

We have elected to characterize the susceptibility of a mouse strain not only by the tumor incidence and latency but also by the carcinogenic index (IBALL, 1939), which equates susceptibility to latency and incidence of tumor induction. It is obvious that certain strains have longer latency periods than others yet produce comparable numbers of tumors. We cannot say at this time whether this represents an inability to respond to certain tumor antigens allowing "sneak through" events to occur or whether these inbred strains actually are capable of mounting a high level of response leading to the blocking phenomena and insuring rapid tumor growth. HALPERN (1973) developed high and low responder lines of Swiss mice to various unrelated antigens. These 2 lines were clearly separated for their humoral responsiveness but showed similar cell mediated reactions. When allogenic Sarcoma 180 implants were made, the high responders allowed the tumor to grow and 90% of the animals were killed, while in the low responders, all tumors regressed. The high responders synthesized high levels of antibody believed to have allowed the tumors to grow due to the blocking reaction, while the low responders produced only enough antibody to provide for effective cellular immunity. If the type C viral antigens play a significant role in chemical carcinogenesis, it may well be that of providing antigenic components in the tumor cell surface which may make them more antigenic as postulated by BARBIERI et al. (1971) and GREENBERGER and AARONSON (1973). On the other hand, a tolerance to these antigens may exist or these endogenous viral antigens may act rapidly to overload the system leading to the blocking phenomena. It is difficult to speculate regarding the wide divergence in antigenicity of chemically induced tumors. It could be assumed, however, that either the individual mice have a wide variation in capability to respond or that the antigenicity of the various chemically induced tumors varies significantly accounting for variations in the immunological response. Such variations in capability to respond would influence the time required for the blocking type phenomena to develop, thereby influencing the rate of tumor development on an individual animal basis (BARTLETT, 1972). The latter of the 2 hypotheses seems most likely since we are dealing with inbred strains, although we have noted a wide variation between individual mice in the mixed lymphocyte studies. We need to define molecular serology of antigens, antibodies, and antigen-antibody complexes in order to study the mechanisms of immune interaction in the mouse model system where the science of immunogenetics is well advanced. Various sensitive serological procedures, such as the radioimmune assay, are available for analyzing the specific antigenic components of the tumor antigens.

Using such studies, we should be able to develop better diagnostic tools and, consequently, a knowledge of the potential usefulness of immunotherapy or immunological preventive procedures without producing an adverse stimulatofy effect to those clones of transformed cells maintained in a "holding" state by host control mechanisms.

Other Genetic Controls of Neoplastic Developments

Many known genes have been associated with the occurrence of various forms of neoplasms; however, in many instances this relationship appears to have no connection with cancer development (HESTON, 1972). Early experiments in cancer research were concerned mainly with the inheritance of susceptibility to spontaneous neoplasia. The development of the inbred strains and the use of congenic strains have allowed for more specific genetic analysis of host susceptibility to carcinogenesis. In most cases, it has been shown that carcinogenesis is dependent on multiple genetic and environmental factors. We will review only a few of the genetic linkages reported to influence cancer development.

HESTON (1963) reported linkage between pulmonary tumors and 8 specific genes (hr, A^y, vt, sh-2, wa-2, Fu, ah, and f), while LITTLE (1934), BITTNER (1945), and HESTON and DERINGER (1948) demonstrated linkage of mammary tumors with lethal yellow, brown, and agouti genes. STRONG (1945) linked gastric tumors with the brown gene while MACDOWELL (1945) and LAW (1952) demonstrated leukemia linked with dilute and flexed-tail genes. Although these associations have been made, their correlation with specific biochemical or physiological pathways has not been made. The H-2 histocompatibility loci has been associated with susceptibility or resistance to leukemia, the $H\text{-}2^k$ being considered susceptible while $H\text{-}2^b$ denotes resistance (LILLY, 1966). How the H-2 locus influences viral leukemia is not known but may represent influences discussed earlier in viral and immunological factor in the development of cancer.

MEIER et al. (1969) have described the genetic control of susceptibility or resistance to viral leukemogenesis by the hairless locus (hr). Hairless is an autosomal recessive mutation maintained in strain HRS/J. The incidence of leukemia is nearly 50% greater in the hairless (hr/hr) mouse than the haired mouse (hr/+) and occurs 6 months earlier. An N-tropic MuLV was isolated from both the hr/hr and hr/+ mice. The latency of neoplastic expression would appear to be related to an immunodeficiency factor rather than infectious virus expression (HEINIGER et al., 1974).

YAMAMOTO et al. (1973) propose that malignancy is induced by carcinogens by producing chromosomal changes resulting in a change in the balance between expression (E) and suppression (S) genes. E exists in normal cells but is neutralized by S, and malignancy occurs only when E increases or S decreases. Their studies with hamster cells injected with polyoma virus or treated with dimethylnitrosamine identified the location of the E and S chromosomes. These studies have been confirmed using Ara-C treatment of hamster cells (BENEDICT et al., 1974).

Cells at Risk to Carcinogens and DNA Repair Synthesis

In addition to the previously discussed genetic factors that provide varying degrees of protection against the initial carcinogenic event, there is an additional factor we wish to discuss: the cells at risk to carcinogenesis. Many factors influence this population of cells, as dose of carcinogens, chronic exposure, site of exposure, aging processes, hormonal influences, stress factors that induce hormonal changes, diet that influences protein metabolism, and promoters such as dust, asbestos, physical, microbiological and chemical irritants, etc. The list is long and cannot be fully exploited here. If, however, one examines this list closely, it is found these factors have one thing in common. They all influence some function of DNA repair. PIERCE (1970) points out that tumors arise only in those tissues capable of mitotic activity. Chemical carcinogens transform only those cells which are entering mitosis which makes up only 0.03% to 0.13% of the body's cells at any one time (MEKLER, 1973). The cells at risk are few and far between unless there are intervening factors that stimulate unscheduled DNA repair. STICH and SAN (1973) indicate a link exists between the oncogenicity of a compound and its capability to provoke DNA repair synthesis. This, however, is not the entire picture, since cocarcinogenic effects do occur requiring both inducers and promoters. Cancer research has made use of this process for years in the form of croton oil in back painting carcinogenesis experiments. This has been carried over into lung carcinogenesis with the use of ferric oxide with BaP (SAFFIOTTI et al., 1968, 1972) and carbon dust or aluminum oxide with BaP (HENRY and KAUFMAN, 1973). STANTON and BLACKWELL (1961) and BLENKINSOPP (1968) stimulated repair and regeneration of pulmonary epithelium by pulmonary infarction. Chronic respiratory infections also promote regeneration of lung tissue and may play a role in carcinogenesis.

The various factors involving DNA repair have not been fully defined. HEINIGER et al. (1972) determined the overall DNA-turnover in 19 inbred strains and F1 hybrid mice. The range of DNA turnover observed suggested polygenic control (H-1, H-3, and H-4) of the steady state. The mouse strains with the shorter turnover time were C57BR/cdJ, DBA/2, SWR/J, and BALB/c. The intermediate turnover rate was represented by C57BL/10, C57BL/6, AKR, C57L, SJL, C58, and C3H/He, while DBA/1 has the longest turnover rate, which was almost 3 times that of C57BR/cdJ. The DNA turnover rate showed no correlation with spontaneous tumorigenesis.

Significant differences in ulcer formation in mice has been observed (LILLY and DURAN-REYNALS, personal communication; NEBERT et al., 1972). The form of host control appears to be primarily that of cells at risk. Much of the body receiving the greatest assault from carcinogenic agents is extracorporeal and has special defense mechanisms. The skin is made up of stratified layers of epithelial cells, with those involved in mitosis being the least exposed to toxic substances. In the respiratory and alimentary systems there is a high degree of vascularization and lymphatic involvement and secretatory activity which tends to produce rapid detoxification and also provides a high level of immunological protection decreasing the incidence of carcinogenic "sneak-through" events occurring. The ciliary action of the respiratory tract also provides for elimination of particulate irritants that induce cellular division.

In the experimental animal models, most chemically induced tumors have had their origin in cells other than the epithilial cells. However,

in lung carcinogenesis, our primary concern is with the induction of squamous cell carcinomas. Alveoli are lined with squamous cells and are suspected to be the site of lung tumors. It is proposed that the use of ferric oxide or other particulate matter or chemicals that will induce mitotic activity increases the cells at risk and may in reality be only an exaggeration of what takes place in nature. Cigarette smoke carcinogenesis may be related to the cocarcinogenesis effects of weak carcinogens and particulate matter requiring extended chronic exposure. We have found that filtered cigarette smoke fails to induce AHH activity in the lungs of mice while unfiltered smoke induced for extended periods (KOURI et al., 1974). The particulate matter appears to play several roles, the trapping of AHH inducers and as promoters for weak carcinogen by increasing cellular proliferation. For these reasons it would appear the use of those agents that bring about unscheduled DNA synthesis are warranted in experimental models. Although we have considered primarily the influence of DNA synthesis on chemical carcinogenesis, this process is also important in triggering the hypothesized depression of repressors of tumor virus genome, protovirus, or oncogenes that may play an integrated role in the host as the etiological agents of cancer, be they viral, chemical or viral-chemical.

Summary and Application in Experimental Pulmonary Carcinogenesis Studies

The selection of animal models for cancer research must be based on the understanding and subsequent characterization of those host regulatory systems that define the differences between susceptible and resistant hosts. We have reviewed such host factors that appear to play decisive roles in carcinogenesis in the inbred mouse as an animal model. Differences in susceptibility to subcutaneous carcinogens with MCA, DMBA, and BaP have been demonstrated between various genotypically different strains of mice. Susceptibility to PAH carcinogens has been shown to be directly related to the inducibility of hepatic AHH, although differences in relative susceptibility to MCA, DMBA, and BaP were demonstrated in AHH inducible strains.

The host control of the type C RNA virus expression was reviewed. Although the frequency of occurrence of "spontaneous" neoplasia has been demonstrated to be related to infectious virus expression in mice, no direct influence of gs antigen or infectious virus expression on chemical carcinogenesis can be demonstrated. Various parameters of immunogenetics were discussed in relationship to the emergence of the initial transformed cells and the ultimate development of cancer. The immunosuppressive effects of carcinogens may play a role in the establishment of transformed cells and their growth. Differences in tumor latency are believed to be related to genetic differences in ability to recognize and respond to the various tumor cell antigens. The full impact of immunogenetics might be further understood when tumor cell antigens are more fully characterized.

We must consider not only the integrated host response but also the cells at risk to chemical carcinogens. Since DNA repair and mitotic activity may increase susceptibility to transformation, this aspect of susceptibility was discussed.

Based on our findings with subcutaneous chemical carcinogens in inbred mice, we have selected several strains for lung carcinogenesis studies. The effects of intratracheal inoculation of chemical carcinogens on AHH induction in the lungs (KOURI et al., these proceedings)

and host immunocompetence (DEMOISE et al., these proceedings) will be correlated with respiratory tumor induction. Studies are also in progress using ferric oxide in combination with chemical carcinogens in hopes of increasing the cells at risk and inducing higher incidences of squamous cell carcinomas. The use of wax pellet carcinogen implants (STANTON and BLACKWELL, 1961) is also being evaluated as a means of inducing lung cancers in mice. By examining these various parameters and methods of tumor induction, we will evaluate the inbred mouse as a model system for lung carcinogens.

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